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RESEARCH ARTICLE

Regulation of HIF-1α and p53 in stress responses in the subterranean rodents *Lasiopodomys mandarinus* and *Lasiopodomys brandtii* (Rodentia: Cricetidae)

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ABSTRACT. The response mechanism and interaction patterns of HIF-1α and p53 in animals in an hypoxic environment are crucial for their hypoxic tolerance and adaptation. Many studies have shown that underground rodents have better hypoxic adaptation characteristics. However, the mechanism by which HIF-1α and p53 in underground rodents respond to hypoxic environments compared with in ground rodents remains unclear. Further, whether a synergy between HIF-1α and p53 enables animals tolerate extremely hypoxic environments is unclear. We studied HIF-1α and p53 expression in the brain tissue and cell apoptosis in the hippocampal CA1 region during 6 hours of acute hypoxia (5% oxygen) in *Lasiopodomys mandarinus* (Milne-Edwards, 1871) and *Lasiopodomys brandtii* (Radde, 1861), two closely related small rodents with different life characteristics (underground and aboveground, respectively), using a comparative biology method to determine the mechanisms underlying their adaptation to this environment. Our results indicate that HIF-1α and p53 expression is more rapid in *L. mandarinus* than in *L. brandtii* under acute hypoxic environments, resulting in a significant synergistic effect in *L. mandarinus*. Correlation analysis revealed that HIF-1α expression and the apoptotic index of the hippocampal CA1 regions of the brain tissues of *L. mandarinus* and *L. brandtii*, both under hypoxia, were significantly negatively and positively correlated, respectively. Long-term existence in underground burrow systems could enable better adaptation to hypoxia in *L. mandarinus* than in *L. brandtii*. We speculate that *L. mandarinus* can quickly eliminate resulting damage via the synergistic effect of p53 and HIF-1α in response to acute hypoxic environments, helping the organism quickly return to a normal state after the stress.

KEY WORDS. Comparative biology, hippocampal CA1 region, oxygen, subterranean rodents.

INTRODUCTION

Oxygen is essential for the metabolism of most living organisms; in particular, it is the basis for the growth and reproduction of aerobic organisms. In many vertebrates, a brief period of lack of oxygen in the brain can irreversibly damage the neurons (Larson et al. 2014). There are many hypoxic environments in the nature, including aquatic habitats (Jackson and Ultsch 2010), high-altitude locations (Qiu et al. 2012), underground burrow systems (Roper et al. 2001), and tumor microenvironments (Gilkes et al. 2014, Spill et al. 2016). Most organisms who have lived in hypoxic environments for a long time have developed several adaptive features. For example, the adaptive characteristics in subterranean rodents are manifested by the convergence of numerous physiological, morphological, behavioral, and genomic features (Šumbera et al. 2004, Kim et al. 2011, Nevo 2013).

Subterranean rodents naturally live in enclosed, hypoxic underground tunnels that present many challenges, including severe hypoxia and limited food availability during extended periods of rain or when the soil freezes during winter. Subterranean rodents have evolved strategies and features to address these environmental obstacles (Shams et al. 2005). For example, during the rainy season, *Spalax carmeli* Nevo, Ivanitskaya & Beiles, 2001 (Rodentia: Spalacidae) can maintain normal life activities in an extremely low-oxygen environment of 7.2% (Shams et al. 2005). Further, *Spalax ehrenbergi* Nehring, 1898 (Rodentia: Spalacidae) can survive for more than 11 hours in an extreme, 3% hypoxic environment, whereas rats can only survive for 2.5 hours in such an environment (Avivi et al. 1999).

In recent years, the molecular mechanisms underlying the response to hypoxia, which includes differential expression of genes encoding hemoglobin, hypoxia-inducible factor-1 (HIF-1), erythropoietin, and vascular endothelial growth factor and the tumor suppressor gene p53, have extensively been studied (Shams et al. 2004, 2005, Schmidt et al. 2017, Dong et al. 2018). Among them, HIF-1, a specific dimer composed of HIF-1α and HIF-1β, regulates the expression of various target genes in a hypoxic environment (Semenza et al. 1997, Zoccola et al. 2017). HIF-1α can bind to p53 and promote p53-dependent cell apoptosis in a hypoxic environment (Suzuki et al. 2001, Zoccola et al. 2017); this may be critical for adaptation to hypoxic environments and inhibit primary tumor growth (Graeber et al. 1994, Nevo et al. 1999, Suzuki et al. 2001, Vousden 2002 Vousden and Lu 2002). Compared with ground rodents, underground rodents, such as the species of *Spalax* (Malik et al. 2012, 2016) as well as *Heterocephalus glaber* Rüppell, 1842 (Rodentia: Bathyergidae) (Park et al. 2017, Husson and Smith 2018), have evolved unique neuroprotective mechanisms for survival in hypoxic conditions. Besides, previous research has demonstrated that *Lasiopodomys mandarinus* (Milne-Edwards, 1871) (Mandarian vole) can adapt to chronic hypoxic environments by increasing oxygen transport capacity and modulating oxygen consumption

(Dong et al. 2018). In addition to being highly adaptable to hypoxic environments, most subterranean rodents, including the naked mole rat, *H. glaber*, and blind mole rat, *Spalax* spp., both of which have strong tumor-suppressing features (Manov et al. 2013, Fang et al. 2014, Gorbunova et al. 2014, Fang et al. 2015). The study on anti-tumor activity in subterranean rodents via molecular biology mainly focused on the regulation and molecular pathways of tumor suppressor genes. p53 can induce cell cycle arrest, repair DNA damage, and promote apoptosis. Both DNA damage repair and apoptosis can reduce the damage caused by hypoxia (such as brain tissue damage) in subterranean rodents. These two pathways are considered the molecular regulation pathways of tumor suppression in subterranean rodents, which are induced by hypoxia or DNA damage (Hammond and Giaccia 2005, Johnson et al. 2005, Morgunkova 2005, Hammond et al. 2006, Prabha et al. 2012).

Lasiopodomys mandarinus is a rodent that lives in chronic hypoxic and dark underground tunnel systems and is widely distributed throughout northeast and central China and north central Mongolia as well as the adjacent areas of Siberia, south of Lake Baikal, and the southern and central Korean Peninsula (Dong et al. 2018, Hong et al. 2019). Oxygen levels in the natural tunnels of these typical subterranean species can reduce to as low as 16.04% in the summer, with further reductions during the rainy season (Hong et al. 2019). This species exhibits remarkable physiological adaptations to hypoxic environments, including increased capillary density and elevated levels of blood parameters such as hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin concentration (Liu et al. 2010). Conversely, *Lasiopodomys brandtii* (Radde, 1861) (Brandt's vole), a species closely related to *L. mandarinus*, is mainly distributed in the grasslands of middle-eastern Inner Mongolia, eastern regions of Mongolia, and some parts of southern Russia (Zhang et al. 2003) and spends most of its life above ground. As *L. mandarinus* and *L. brandtii* have a close evolutionary relationship and distinct life histories, they are ideal animal models for comparatively studying the mechanisms underlying the adaptation to hypoxia in subterranean mammals.

Brain tissues are extremely susceptible to hypoxia; hypoxic damage to the nervous system and brain tissues is often irreversible. For example, hypoxia can induce cerebral vascular stenosis or even occlusion, hindering oxidative metabolism and energy supply; this results in permanent damage to the nervous system and brain parenchyma. In addition, hypoxia can lead to the accumulation of specific metabolites, such as vasopressin, in tissues, resulting in increased intracranial pressure, brain edema, severe cell metabolism disorders, and brain atrophy (Terraneo and Samaja 2017). Therefore, if a hypoxia-intolerant animal is exposed to hypoxia, its nervous system and brain development may be affected. In addition, the hippocampal CA1 region, an important structure in the limbic system of the brain, is significantly related to the cognition and learning ability of animals and is very sensitive to external environmental stresses, such as hypoxia. Therefore, in the present study, we exposed the voles to acute hypoxic environmental stress (5% oxygen) for 6 hours and measured the expression levels of HIF-1α and p53 in their brain tissues and the apoptosis index in the hippocampal CA1 region during the following month of normal oxygen levels in order to explore how HIF-1α and p53 help the two types of voles to respond to acute hypoxic exposure and whether the two have a synergistic effect. Further, we investigated the differences in the response mode of HIF-1α and p53 in the two voles and whether such difference helps the animals tolerate extremely hypoxic environments.

MATERIAL AND METHODS

Lasiopodomys mandarinus was trapped from croplands in Xinzheng, Henan, China (N 34°52', E 113°85'); *L. brandtii* was obtained from the Chinese Academy of Agricultural Science. They were maintained in polycarbonate cages (37×26×17 cm^3) on a 14:10 hours light/dark cycle at 20–24 °C for at least one month. Rat and rabbit feed (produced by Henan experimental animal center, Zhengzhou, China) with fresh carrots was made regularly available during feeding periods. According to their typical social systems, *L. mandarinus* were monogamously raised and *L. brandtii* were raised in groups. The two vole species were housed in separate rooms to prevent odor interference. All animals were maintained in the laboratory at the School of Life Sciences, Zhengzhou University, for more than one generation.

To mimic acute hypoxic environmental stress, 72 three month old healthy adult male voles (n = 36 of each species) were randomly divided into the following six groups (n = 6 of each species): normoxia (20.9% oxygen for 6 hours), hypoxia A (5% acute

hypoxia for 6 hours with no oxygen restoration), hypoxia B (5% acute hypoxia for 6 hours followed by 12 hours of normal oxygen levels), hypoxia C (5% acute hypoxia for 6 hours followed by 24 hours of normal oxygen levels), hypoxia D (5% acute hypoxia for 6 hours followed by 14 days of normal oxygen levels), and hypoxia E (5% acute hypoxia for 6 hours followed by 28 days of normal oxygen levels). Individuals in the normoxic group were also placed in an unopened oxygen chamber for 6 hours. Except for the oxygen content, all other environmental conditions remained the same for all groups. Acute hypoxia was simulated using a DS-II hyperbaric cabin (Huaxin Hyperbaric Cabin, Weifang, China). The oxygen level in the cabin was maintained at a constant level by balancing oxygen and nitrogen flow rates and was monitored using an oximeter. A bottle containing sodium hydroxide was placed in the cabin to absorb the carbon dioxide released by the animals. All experiments were conducted in the morning to avoid the effects of different circadian rhythms on the animals. Immediately after treatment completion, the animals were sacrificed via an overdose of pentobarbital sodium (30 mg/kg). The brain tissues of the experimental animals were quickly removed and the CA1 region was quickly separated from the hippocampus. Whole-brain tissues were placed in an ultra-low temperature freezer at -80 °C until RNA extraction by grinding the tissues in liquid nitrogen. On the other hand, the CA1 regions were fixed in 4% paraformaldehyde and stained using the TUNEL (Roche, China) method.

Total RNA was extracted from the brain tissue of each animal using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. Residual DNA was removed by treatment with RNase-free DNase I (Takara Bio, Dalian, China). RNA integrity was verified via agarose gel electrophoresis (1.2%), and RNA concentrations were measured using an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

The total RNA extracted from the brain tissues was reverse-transcribed into cDNA using a reverse transcription kit (Takara Bio). The obtained cDNA was stored at -20°C for the subsequent real-time PCR.

The HIF-1α and p53 sequences of the near relatives of *L. mandarinus* were determined and compared with their corresponding sequences in the National Center for Biotechnology Information before designing their primers for real-time PCR usingthe Primer3 online software (Rozen and Skaletsky 1999, Schmittgen and Livak 2008). The primers were further verified via sequencing after the PCR products were assembled into cloning vectors. The details of the primers are shown in Table 1.

Real-time PCR was performed on a Rotor-gene 3000 fluorescence quantitative PCR instrument (Corbett Research, Germany) with a default reaction procedure configuration. The reaction volume was 20 μL and included 0.5 μL each of specific forward and reverse primers (20 μM), 2 μL of the diluted cDNA template, 1 μL of the PrimeScript RT Enzyme Mix, 6 μL of the 5× PrimeScript Buffer, and 10 μL of RNase-free H_2O. β-actin was used as the internal control gene (forward: GTCGTACCACTGGCATTGTG; reverse: CCATCTCTTGCTCGAAGTCC), and relative gene expression was determined using the comparative CT method (Untergasser et al. 2012).

Paraffin sections of the brain tissue were prepared after fixing the brain tissue of each animal in paraformaldehyde. These dewaxed and hydrated brain tissues were stained using a TUNEL method (Lozano et al. 2009). Cells with brown-yellow granules in the nucleus were identified as positive apoptotic cells under a microscope (Leica DMI8M, Germany). For each TUNEL-stained section, five fields of view were randomly selected, and the percentage of apoptotic cells in each field was calculated at high magnification (×400).

All data were analyzed using SPSS (version 22.0, SPSS Inc., USA) and presented as mean ± SE. $p < 0.05$ denoted statistical significance. The data were initially checked for homogeneity of variance. If appropriate, the statistical significance between different groups was assessed using two-way ANOVA followed by Duncan's post-hoc tests. If heterogeneity of variance existed between the groups, the Kruskal–Wallis test followed by the Mann–Whitney U post-hoc test was used for comparing the differences between the groups (Buchley 2006).

The experimental protocol was approved by the Animal Care and Use Committee of Zhengzhou University (Approved project number: 31372193) and in accordance with the Guide for the Care and Use of Laboratory Animals of China.

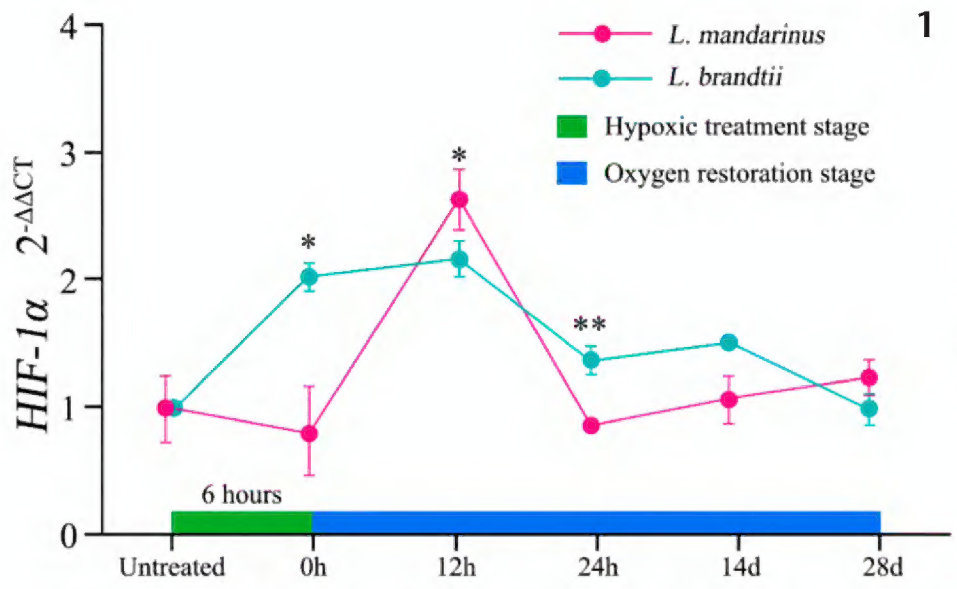
RESULTS

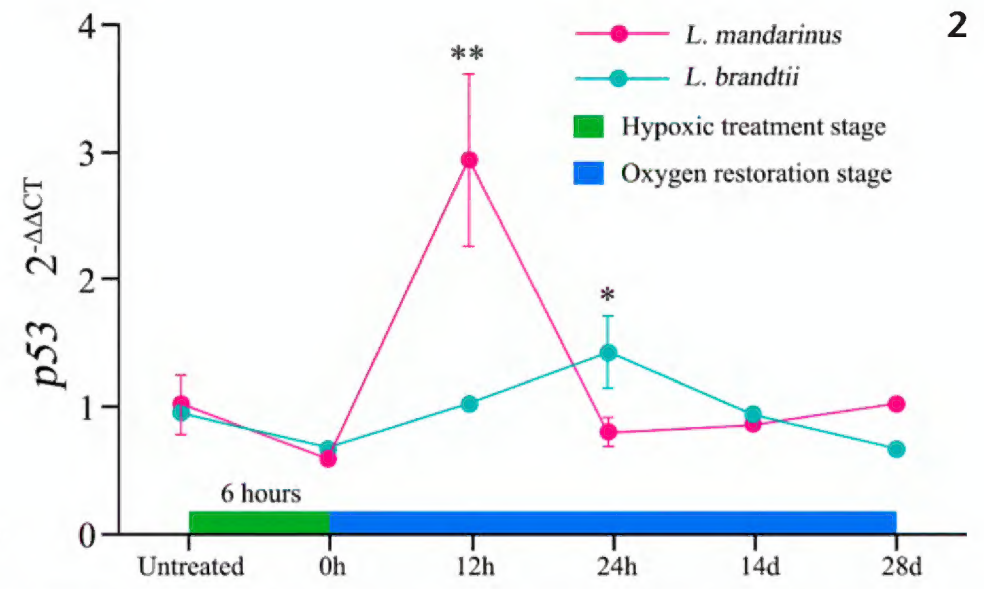
Changes in the mRNA expression levels of HIF-1α and p53

During the entire experiment, the expression level of HIF-1α in both the brain tissues of both voles tended to first increase before subsequently decreasing, and its expression peaked in both voles at 12 hours after oxygen restoration (Fig. 1). The expression of HIF-1α in the brain tissue of *L. mandarinus* was significantly higher than that in the brain tissue of *L. brandtii* at 12 hours after oxygen restoration ($p = 0.043$), whereas the expression of HIF-1α was significantly lower in the brain tissue of

Table 1. Details of the primers used.

Gene name	Sequences	Length (bp)	Annealing temperature
HIF-1α	Forward: AGTTCTGAACGTCGAAAAG Reverse: CAGGATCAGCACTACTTCG	1287 bp for *L. mandarinus* 2173 bp for *L. brandtii*	55°C
p53	Forward: CCCCTGTCATCTTTTGTCCCT Reverse: GCTGGCAGAATAGCTTATTGAG	1176 bp for *L. mandarinus* 1177 bp for *L. brandtii*	55°C





Figures 1–2. Relative mRNA expression levels of HIF-1α (1) and p53 (2) in the brain tissues *of Lasiopodomys mandarinus* and *L. Brandtii* before and after the hypoxic treatment and at different reoxygenation times. The X-axis represent the different time points during hypoxic treatment and reoxygenation. The Y-axis represent the relative expression levels of HIF-1α and p53, respectively, during these time points.

L. mandarinus than in that of *L. brandtii* at 0 and 24 hours after oxygen restoration ($p = 0.025$ and $p = 0.002$, respectively) (Fig. 1).

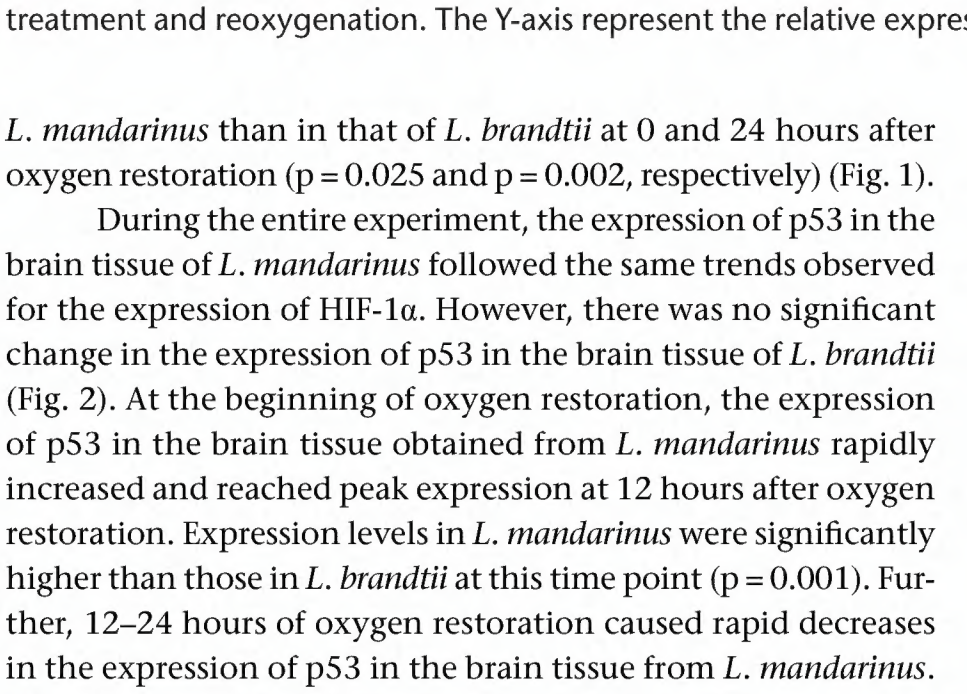
During the entire experiment, the expression of p53 in the brain tissue of *L. mandarinus* followed the same trends observed for the expression of HIF-1α. However, there was no significant change in the expression of p53 in the brain tissue of *L. brandtii* (Fig. 2). At the beginning of oxygen restoration, the expression of p53 in the brain tissue obtained from *L. mandarinus* rapidly increased and reached peak expression at 12 hours after oxygen restoration. Expression levels in *L. mandarinus* were significantly higher than those in *L. brandtii* at this time point ($p = 0.001$). Further, 12–24 hours of oxygen restoration caused rapid decreases in the expression of p53 in the brain tissue from *L. mandarinus*. After 24 hours of oxygen restoration, the expression of p53 in the brain tissue from *L. brandtii* was significantly higher than that in the brain tissue from *L. mandarinus* ($p = 0.033$).

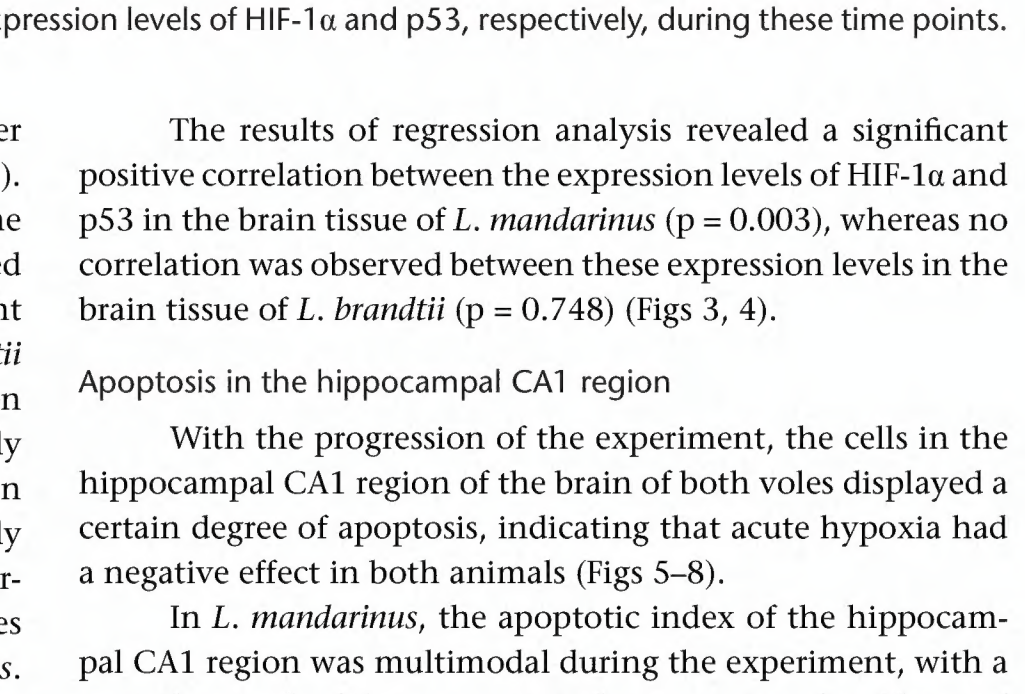
The results of regression analysis revealed a significant positive correlation between the expression levels of HIF-1α and p53 in the brain tissue of *L. mandarinus* ($p = 0.003$), whereas no correlation was observed between these expression levels in the brain tissue of *L. brandtii* ($p = 0.748$) (Figs 3, 4).

Apoptosis in the hippocampal CA1 region

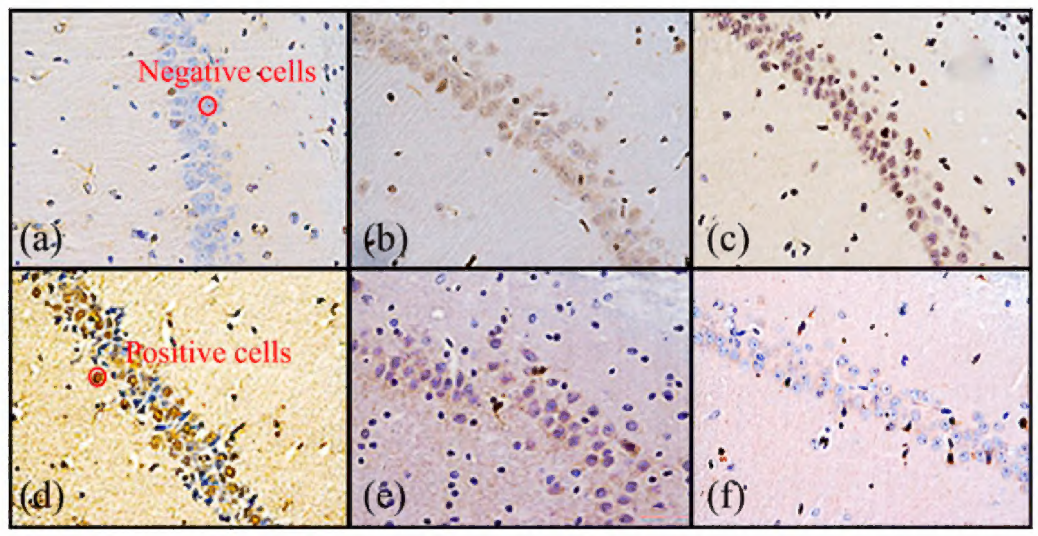
With the progression of the experiment, the cells in the hippocampal CA1 region of the brain of both voles displayed a certain degree of apoptosis, indicating that acute hypoxia had a negative effect in both animals (Figs 5–8).

In *L. mandarinus*, the apoptotic index of the hippocampal CA1 region was multimodal during the experiment, with a secondary peak of the apoptotic index occurring after 0 hour of oxygen restoration, and the main peak occurring after 24 hours of oxygen restoration (Fig. 5). In *L. brandtii*, the apoptotic index





Figures 3–4. Regression analysis of the relative mRNA expression levels of HIF-1α and p53 in the nrain tissues of *Lasiopodomys mandarinus* (3) and *L. brandtii* (4). The X-axis and Y-axis represent the relative mRNA expressions levels of HIF-1α and p53, respectively.



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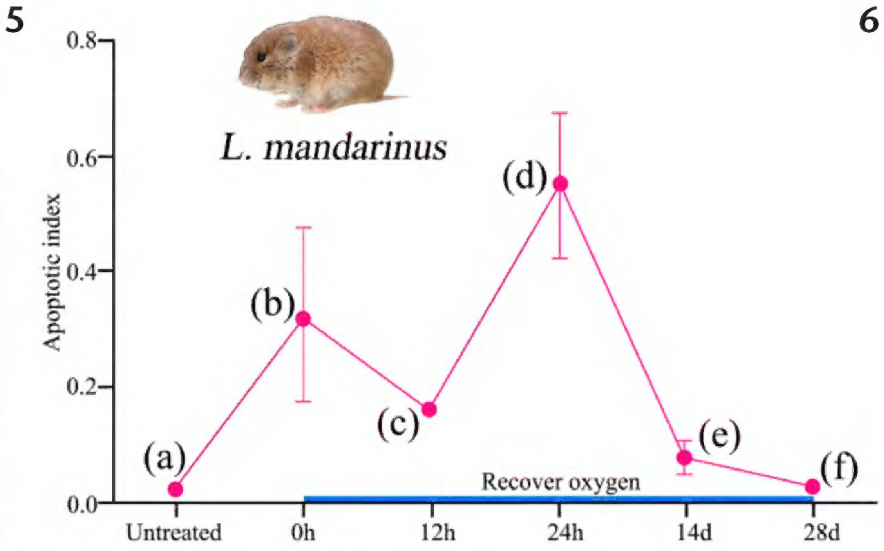

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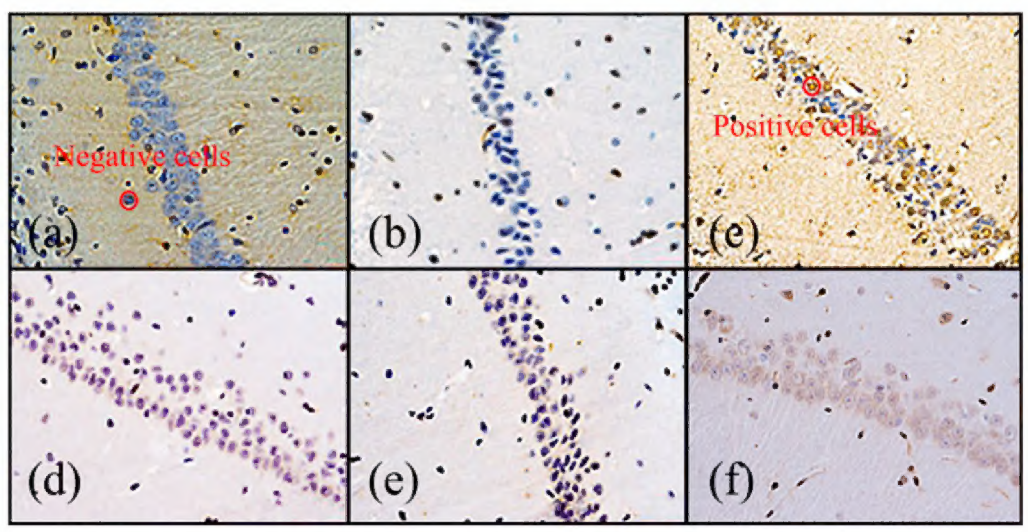
Figures 5–6. Microscopic observation of the apoptosis in the hippocampal CA1 region (5) and a broken line diagram of the apoptotic index (6) of the brain tissue of *Lasiopodomys mandarinus* at different time points after oxygen restoration. (5) The microscopic images of the apoptosis in the hippocampal CA1 region of *L. mandarinus*. Cells with a yellow nucleus are apoptotic, whereas cells with a blue nucleus are normal. (6) The apoptotic index (broken line) of the brain tissue of *L. mandarinus* at different time points after oxygen restoration ("Reover oxygen"). The X-axis represents different time points during hypoxic treatment and reoxygenation, and the Y-axis represents the apoptosis index of the hippocampal CA1 region corresponding to specific time points.



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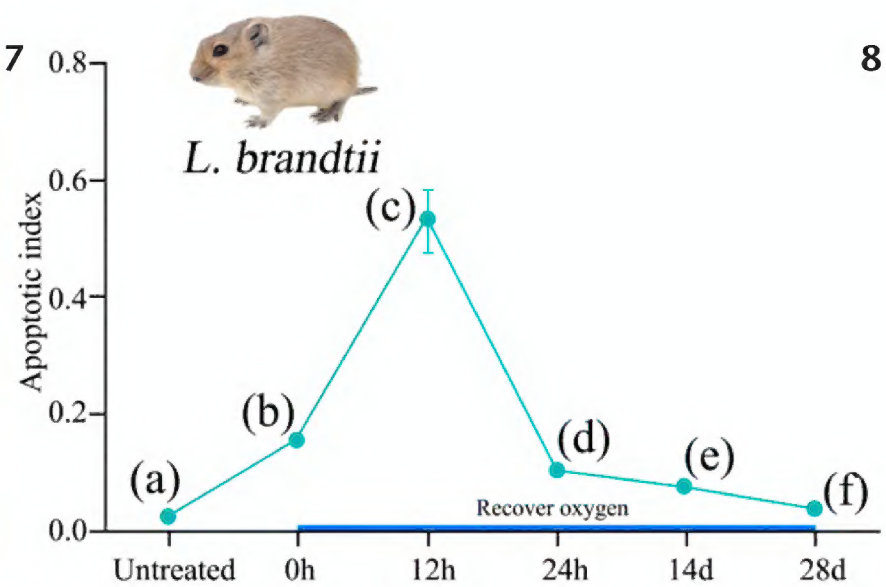

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Figures 7–8. Microscopic observation of the apoptosis in the hippocampal CA1 region (7) and a broken line diagram of the apoptotic index (8) of the brain tissue of *Lasiopodomys brandtii* at different time points after oxygen restoration. (7) Cells with a yellow nucleus are apoptotic cells, whereas cells with a blue nucleus are normal cells. (8) The X-axis represents different time points during hypoxic treatment and reoxygenation, and the Y-axis represents the apoptotic index of the hippocampal CA1 region corresponding to these time points.

of the hippocampal CA1 region was unimodal, peaking after 12 hours of oxygen restoration (Fig. 8). At 12 hours after oxygen restoration, the apoptosis index in the hippocampal CA1 region was significantly higher in *L. brandtii* than in *L. mandarinus* ($p = 0.001$). However, at 24 hours after oxygen restoration, the apoptosis index was significantly higher in *L. mandarinus* than in *L. brandtii* ($p = 0.002$).

Correlation between the expression of HIF-1α and the apoptosis index

There was a significant negative and positive correlation between the expression level of HIF-1α and the apoptotic index of the hippocampal CA1 region of the brain tissue of *L. mandarinus* ($p = 0.008$; supplementary Fig. S1) and *L. brandtii* ($p = 0.005$; supplementary Fig. S1), respectively, under hypoxia.

DISCUSSION

Fluctuations in the expression of HIF-1α

In this study, we showed that while the mRNA expression level of HIF-1α in both voles peaked at the same time; however, the overall trend of its expressions differed. Our results suggest that the expression level of HIF-1α in the brain tissue of *L. mandarinus* responded to changes in oxygen levels and recovered more rapidly than that in the brain tissue of *L. brandtii*.

HIF-1 is the primary regulator to maintain the body's oxygen homeostasis in mammals, including humans, In addition. it is a heterodimer comprising an unstable HIF1-α subunit and structural protein HIF1-β subunit. The HIF1-α subunit is the main effector of hypoxic response (Valle-Tenney et al. 2020, Urrutia and Aragonés 2018). Under normoxic conditions, the

two conserved proline residues of HIF1-α present in the cytoplasm are hydroxylated by enzymes (PHD family) containing the HIF-prolyl hydroxylase domain. Hydroxylated HIF1-α is recognized and degraded by E3 ubiquitin ligase, which containsvon Hippel–Lindau tumor suppressor protein (Robert et al. 2001). However, hypoxia inhibits the activity of the PHD family proteins and prevents the hydroxylation of HIF1-α. The accumulated HIF1-α translocates to the nucleus and heterodimerizes with the HIF1-β subunit. The complex of HIF1-α and HIF1-β binds to DNA at the hypoxia response element to promote the transcription of downstream target genes in order to maintain the body's oxygen homeostasis (Kaelin and Ratcliffe 2008, Urrutia and Aragonés 2018).

Hypoxia activates the PI3K/Akt pathway and the ERK pathway, which play a role in promoting the stability of HIF1-α and activating the transcriptional activity of HIF1-α, respectively (Minet et al. 2001). Under the activated transcription of HIF1-α, the expression of EPO (encoding erythropoietin) increases, stimulating the proliferation and differentiation of red blood cells to enhance the oxygen transport capacity in tissues (Wang and Semenza 1995). Further, the expression of vascular endothelial growth factor, which induces angiogenesis and vasodilation to increase the transport of oxygen and nutrients, is increased (Basic et al. 2019, Li et al. 2020). Moreover, the expression of various genes encoding glycolytic enzymes, including ALDA (encoding aldol Enzyme A), Ldha (encoding lactate dehydrogenase A), and ENO1 (encoding enolase 1), is increased, promoting glycolysis to make up for the lack of energy production in the body (Semenza et al. 1996).

Lasiopodomys mandarinus spends its entire life in underground tunnels, and it has good adaptability to hypoxic environments (Dong et al. 2018, Hong et al. 2019), which could be one reason why the expression levels of HIF-1α in its brain tissue did not significantly change during the 6 hours of hypoxia. Prior studies have indicated that in extremely low-oxygen environments, a series of tissues and organs in *L. mandarinus* can significantly reduce oxygen consumption to ensure oxygen supply to the brain and other important organs (Dong et al. 2018). In addition, previous research has shown that extreme hypoxic environments change the permeability of the blood-brain barrier in subterranean mammals. In subterranean mammals, blood-brain barrier permeability is stronger in response to hypoxia than in response to normoxia. When the oxygen levels in the environment normalize, oxygen in the blood enters the brain more quickly, allowing the hypoxic state to be quickly eliminated, thereby protecting the brain tissue (Kondo et al. 1996, Brown et al. 2003 Deli et al. 2005). In our study, the expression of HIF-1α fluctuated more rapidly in the brain tissue of *L. mandarinus* than in that of *L. brandtii*, returning to normal levels after 24 hours of oxygen restoration in *L. mandarinus*, whereas taking until 28 days after oxygen restoration to reach normal levels in *L. brandtii*. Therefore, we hypothesized that a more sensitive HIF1-α expression pattern could help *L. mandarinus* respond more actively and flexibly to the hypoxic environment than *L. brandtii*.

Fluctuations in the expression of p53

The current study indicates that hypoxic stress causes an increase in damaged cells in both vole species. After normal brain tissue oxygen levels are restored, the DNA of the damaged neurons will bind to the hydrophobic region of p53 and repair itself, and if the cells are too damaged to repair themselves in the hypoxic environment, p53 can induce apoptosis (Oda et al. 2000, Suzuki et al. 2001, Fang et al. 2005). After the clearance of all damaged cells, the expression of p53 gradually decreases to the normal level (Suzuki et al. 2001). To some extent, regulation of the expression of p53 is a protective mechanism of the body.

As a tumor suppressor, p53 plays important roles in regulating cell cycle, cell apoptosis, and DNA damage repair (Madan et al. 2019). Under normal conditions, p53 is maintained at a minimal level via continuous degradation by E3 ubiquitin ligase Mdm2 (Sermeus and Michiels 2011). However, under hypoxic conditions, p53 is activated through a complex mechanism. The direct interaction of HIF1-α and Mdm2 inhibits the Mdm2-mediated degradation of P53. Further, HIF1-α induces the protein phosphatase 1 nuclear targeting subunit to promote p53phosphorylation and prevent its degradation by Mdm2. In addition, the accumulated p53 is further activated by inducing the degradation of HIF1-α; acetylation fully activates p53 (Wang et al. 2019). The activated p53 activates the transcription of downstream target genes involved in DNA damage repair, cell cycle, and apoptosis (Pan et al. 2004). For example, p53 prevents cell cycle progression by activating the expression of cell cycle inhibitors P21 and 14-3-3σ (Paris et al. 2008), inducing apoptosis by activating the expression of Apaf1, BNIP3L, Bax, Puma, Noxa, and Perp (Feng et al. 2011, Shams et al. 2013), initiating DNA damage repair by inducing DDB2 and XPC (Hafner et al. 2019).

Mutations in the p53 gene usually worsens tumor conditons. However, mutations in the P53 gene in some underground animals living in hypoxic environments not only promote the occurrence of tumors. but also enhance the animals' ability to adapt to hypoxia by regulating the cell cycle and apoptosis. Two mutations in p53's DNA binding domain in *Spalax* lead to the enhanced transcription of target genes that inhibit cell cycle, including Mdm2, Pten, CycG, and p21; the loss of function of apoptosis-related genes, including Apaf1, Puma, Noxa, and Bax; and an increase in the level of Apip, which inhibits apoptotic genes downstream of p53 in a hypoxic environment. However, in rats, the expression patterns of Apip, Apaf1, and Mdm2 are all opposite to those in *Spalax* (Band et al. 2010, Shams et al. 2013). Therefore, cell cycle arrest and stalled activation of apoptosis caused by TP53 mutations may be a unique mechanism of action of *Spalax* and other subterranean rodents adapting to an extremely hypoxic environment (Ashur-Fabian et al. 2004).

In our study, the expression of p53 in brain tissue from *L. mandarinus* exhibited a dramatic oscillation, wherein it increased

initially and then decreased. This pattern was highly consistent with changes in the expression of HIF-1α in vivo, whereas the patterns in *L. brandtii* were not. Previous studies have shown that under severely hypoxic conditions, accumulated p53 binds to the ODD domain of HIF1-α, promoting the degradation of HIF1-α through Mdm2-mediated ubiquitination; this results in the inhibition of the effect of HIF1 in promoting angiogenesis (Sánchez-Puig et al. 2005, Hansson et al. 2002). However, the relationship between p53 and HIF-1α is still not fully understood. The study by Madan et al. in cancer cells showed that the combination of p53 and HIF1-α enhanced the transcriptional activity of HIF1 and increased the expression of the downstream genes of HIF1, thereby increasing cell survival in a hypoxic environment (Madan et al. 2019). At present, there is little research on how p53 and HIF1-α cooperate in the hypoxic environment in underground rodents. Our study proposes that HIF-1α and p53 in *L. mandarinus* interact during the initial reoxygenation phase and induce functions related to DNA repair and injury prevention, cell cycle arrest, and apoptosis (p53 signaling pathway). Our interpretation is supported by the significant positive correlation between the expression of HIF-1α and p53 in *L. mandarinus* (Figs 3, 4).

Apoptosis in the hippocampal CA1 region

Apoptosis and other injuries caused by ischemia and hypoxia in rodents are generally manifested 2 hours after oxygen restoration (Li et al. 1995). Conversely, in the present study, we observed that apoptosis was induced in the brain tissue of both voles during hypoxia. Considering that *S. carmeli*, a typical subterranean rodent species, has the lowest oxygen content (7.2%) in its natural environment (Shams et al. 2005), it was speculated that 5% oxygen content is much lower than that experienced by the two experimental voles in nature, which may causes acute damage to the body.

During the 28-day period of oxygen restoration, cells in the hippocampal CA1 region of both voles demonstrated certain degrees of fluctuation. The peak apoptotic index of both species was between 55% and 60% (Figs 5–8). However, within 24 hours of oxygen recovery, the apoptotic index of the hippocampal CA1 region of *L. mandarinus* decreased and then increased. Conversely, it increased and then decreased in *L. brandtii*. It is noteworthy that at 12 hours of reoxygenation, the apoptosis index in the hippocampal CA1 region of *L. mandarinus* was significantly lower than that of *L. brandtii*, whereas the expression of p53 in the brain tissue from *L. mandarinus* was significantly higher than that in the brain tissue from *L. brandtii* at the same time point. These findings suggest that in the early reoxygenation stage, decreases in the apoptotic index of the brain tissue from *L. mandarinus* are probably related to the high expression levels of HIF-1α and p53. Moreover, this result is consistent with the speculation that hypoxic environments induce the high expression levels of HIF-1α and p53 in the brain tissue from *L. mandarinus* to partially alleviate damage to the body.

Compared with *L. mandarinus*, HIF-1α did not successfully induce a high expression of p53 in the body of *L. brandtii*, and thus, this gene may not inhibit apoptosis of cells in the brain tissue. Previous studies have shown that because of long-term adaptation of subterranean mammals to hypoxic environments, their bodies usually respond to hypoxic environmental stress by inhibiting cell activity, promoting cell cycle arrest, increasing oxygen transport capacity, and modulating oxygen consumption (Fang et al. 2014, Xiao et al. 2017, Dong et al. 2018). The present study indicates that the high expression of p53 induced by HIF-1α may be a unique strategy for subterranean rodent groups to adapt to low-oxygen levels by which body damage is reduced by regulation of damaged DNA repair.

In conclusion, our results suggest that because of long-term existence in underground burrow systems, better adaptation to hypoxia is observed in *L. mandarinus* than in *L. brandtii*. Under acute hypoxic environmental stress, *L. mandarinus* can quickly cope with body damage through the cooperative effect of p53, HIF-1α, permitting rapid return to a normal state after environmental stress elimination. These results help us better understand the response patterns of p53 and HIF-1α in underground rodents under a hypoxic environment. However, the specific mode of regulation and underlying mechanism of p53 and HIF-1α in subterranean rodents under a hypoxic environment requires further in-depth study.

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SUPPLEMENTARY MATERIAL

Supplementary material 1

Figure S1. Regression analysis results of the relative expression level of HIF-1α and the apoptotic index of the hippocampal CA1 region in the brain tissues of *L. mandarinus* (A) and *L. brandtii* (B).

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